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Transduction in *Bacillus cereus* and *Bacillus anthracis*

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INTRODUCTION

A phage, designated CP-51, that carries out generalized transduction in *Bacillus cereus* and *B. anthracis*, has recently been isolated from soil. Transducing phages for members of the genus *Bacillus* have been described previously for *B. subtilis* and *B. licheniformis* (2-6). Those that have been best characterized are PBS-1 for *B. subtilis* (4) and SP-10 and SP-15 for both *B. subtilis* and *B. licheniformis* (5, 6). None of these transducing phages is active on *B. cereus* or *B. anthracis*. Thus, the availability of CP-51 broadens the range of organisms in which genetic exchange is now possible.

MATERIALS AND METHODS

B. cereus NRRL 569 was the strain used as indicator for isolation of CP-51, and transduction was first demonstrated in auxotrophic mutants derived from that strain. Details of the procedures involved in those studies have been published (7). The studies were extended to include other strains of *B. cereus* and *B. anthracis*, and details of these experiments will be reported later.

Phage was grown by the soft-agar layer method of Adams (1), and lysates were filtered (through AA membranes; Millipore Corp., Bedford, Mass.) to remove bacterial cells. The number of plaque-forming units (PFU) per ml was determined by the agar layer method (1). The medium used for phage assays was PA agar, composed of Difco Nutrient Broth and salts at *p*H 5.9. Spores of 569 were used as indicator inoculum for routine assays, but spores of other strains were used when appropriate.

Transductions were carried out by incubating mixtures of recipient cells and phage on a shaker at 37 C for 30 min. Samples were plated on minimal medium for scoring transductants. All cultures were tested for revertants, and all phage preparations were tested for bacterial contamination.

Two minimal media were used for scoring transductants. Medium M10 (8) contained, in addition to salts, glucose, and glutamic acid, the following six amino acids: alanine, serine, threonine, valine, leucine, and isoleucine. In medium M10C, these six amino acids were replaced by casein hydrolysate.

Cells for transduction were usually grown in NBY broth composed of Difco Nutrient Broth and Difco yeast extract. In this medium, the organisms grew in chains of variable length, and the values for viable counts represent numbers of chains or colony-forming units rather than numbers of individual cells.

The following abbreviations are used in designating phenotypes: Trp, tryptophan; His, histidine; Met, methionine; Ile, isoleucine; Leu, leucine; Gly/Ser, glycine or serine; +, independent; −, dependent.

RESULTS AND DISCUSSION

The Phage

CP-51 formed very distinct plaques on both NBY and PA media. With strain 569 as the indicator, the efficiency of plating was about 30% higher on PA than on NBY, and for that reason PA was used routinely for assays. The plaques normally had a small colony in the center, but mutants that gave completely clear plaques occurred spontaneously. When colony-centered plaques were the source of inoculum for phage propagation, preparations usually had low proportions of clear PFU. Phage yields of 10^{11} to 2.5×10^{11} were obtained routinely with strain 569 as the host.

CP-51 particles have the form of a head with a

contractile tail. Details of the phage structure as revealed by electron microscopy will be reported later.

Host Range

Table 1 shows the results of testing several strains of *B. cereus* and various other species as hosts for growth of CP-51. Of seven strains of *B. cereus* tested, four served as hosts for propagation and three did not. *B. thuringiensis* and the Sterne strain of *B. anthracis* also served as hosts. The other species tested, *B. subtilis*, *B. brevis*, *B. megaterium*, and *B. licheniformis*, were ineffective.

Stability of CP-51

CP-51 has unusual stability characteristics. Preparations that were stored in a refrigerator (2 to 4 C) lost up to 90% of their initial numbers of PFU in 24 hr. Experiments devised in an arbitrary manner to find methods to stabilize the phage revealed that (i) the stability was enhanced by diluting preparations 1:5 or more in NBY broth, and (ii) preparations were considerably more stable at room temperature (24 to 28 C) than at 2 to 4 C. When tests were done to determine the optimal temperature for maintenance of PFU, the results shown in Table 2 were obtained. Of those temperatures tested, 15 C was the most satisfactory. Temperatures between 4 and 15 C or between 15 and 21 C have not been tested.

Dilution of the phage in NBY broth and storage at 15 C offered an arbitrary but convenient way of sufficiently stabilizing phage preparations for use in transduction tests; therefore, attempts to elucidate the factors involved in stabilization were postponed. Future plans for research on CP-51 include a detailed investigation with respect to its stability characteristics.

Because CP-51 was unstable, a primary source of it was maintained in the form of infected 569 spores. When plated for infected centers by the phage assay procedure, such spores served as a convenient source of plaques for further propagation.

TABLE 1. *Host range of CP-51*

Organism	Reaction[a]
B. cereus NRRL 569	+
B. cereus T	+
B. cereus ATCC 6464	+
B. cereus ATCC 9139	+
B. cereus ATCC 9592	−
B. cereus ATCC 7004	−
B. cereus ATCC 11950	−
B. thuringiensis NRS 1328	+
B. anthracis, Sterne	+
B. subtilis W-23	−
B. brevis ATCC 8185	−
B. megaterium ATCC 11561	−
B. licheniformis ATCC 9945A	−

[a] Plus signs indicate that the strain served as host; minus signs indicate no detectable activity.

TABLE 2. *Stability of CP-51 at various temperatures*[a]

Storage temp (C)	Percentage of initial PFU remaining after		
	4 days	15 days	58 days
2–4	20	2.5	0.2
15	100	100	33
21	100	92	2
29	52	16	0.02
37	15	<0.001	

[a] Preparation was diluted 1:10 in NBY broth, and samples were stored at the given temperatures.

TABLE 3. *Inhibition of transduction by phage antiserum and heat*[a]

Treatment of phage	Trp^+ transductants/ml
Phage omitted	0
No treatment	830
65 C, 30 min	0
Incubated with phage antiserum	0
Incubated with normal serum	780

[a] Transduction mixtures contained 0.8 ml of RM20 (Trp^-) cells (5×10^8) and 0.1 ml of phage (7.2×10^8 PFU) propagated on wild-type 569. In the tests with serum, phage was incubated with 0.1 ml of normal serum or phage antiserum for 15 min at 37 C before cells were added. In the other mixtures, 0.1 ml of NBY broth was added so that the final volume of each was 1.0 ml. The treatment with antiserum inactivated 99.99% of the PFU, and no PFU could be detected in the heated sample. Transductants were scored on M10C.

Transduction in Strain 569

The data in Table 3 provide evidence for transduction of a Trp^- mutant of 569 to Trp^+ by phage propagated on wild-type 569. From the mixture containing cells and phage, 830 transductants per ml were obtained. In the absence of phage, no colonies were produced on the minimal medium. Inactivation of phage by incubation with phage antiserum or by heating at 65 C for 30 min prevented transduction. Further evidence that the change from auxotrophy to prototrophy was indeed transduction is pro-

vided by the data in Table 4. In the experiment shown, phage propagated on a Trp⁻ mutant failed to induce prototrophy in cells of the same mutant, although this phage was as effective as phage propagated on wild type in transducing His⁻ and Met⁻ mutants. The data in Tables 3 and 4, along with the fact that treatment of phage preparations with deoxyribonuclease did not reduce their effectiveness in inducing the change from auxotrophy to prototrophy, provide firm evidence for transduction.

Several factors affected the yield of transductants; among them were (i) the medium used for scoring, (ii) the multiplicity of infection, (iii) age of recipient cells, (iv) the proportion of clear PFU in phage preparations, and (v) differences in susceptibility of recipient cells of various mutants to lysis or killing by the phage. In general, the yield of transductants was increased by the use of phage that was treated with ultraviolet light (UV) to inactivate 99% or more of the PFU. With UV-treated phage, the recovery of transductants was enhanced still further in many instances by plating samples in the presence of phage antiserum. However, with UV-treated phage and a Trp⁻ mutant of 569, for example, plating on M10C in the presence of antiserum sometimes caused a reduction in the yield of transductants. A possible explanation for this is that adsorption of phage continued to occur after transduction mixtures were plated, and the presence of antiserum would prevent such further

TABLE 4. *Specificity of transduction by phage propagated on an auxotrophic mutant*[a]

Recipient cells	Colonies/ml		
	Without phage	With phage propagated on	
		Wild type	RM20 (Trp⁻)
RM20 (Trp⁻)	0	620	0
RM1 (His⁻)	10	400	640
RK36 D60 (Met⁻ Ile⁻)	0 (Met⁺)	470 (Met⁺)	440 (Met⁺)

[a] Each phage preparation had 1.7×10^{10} PFU/ml before UV treatment that inactivated 99.1% of the PFU. Transduction mixtures consisted of 0.9 ml of cells (about 6×10^8) and 0.1 ml of phage or NBY broth. Transductants for Trp were scored on M10C, and those for His and Met were scored on M10.

TABLE 5. *Transduction by UV-irradiated phage*[a]

Transduction mixture		PFU inactivated by UV (%)	Colonies/ml when plated	
Recipient	Multiplicity of infection		Without antiserum	With antiserum
RM20 (Trp⁻)	0	—	10	0
	1.4	0	880	1,200
	1.4	93	1,080	1,250
	1.4	99.1	1,140	1,060
	1.4	99.9	780	840
RK36 D60 (Met⁻ Ile⁻)	0	—	20 Met⁺, 20 Ile⁺	5 Met⁺, 20 Ile⁺
	3	0	10 Met⁺, —[b]	620 Met⁺, —
	3	99.1	120 Met⁺, —	640 Met⁺, —
	1.4	99.1	570 Met⁺, 0 Ile⁺	1100 Met⁺, 680 Ile⁺
RK36 D11 (Met⁻ Leu⁻)	0	—	5 Met⁺, 20 Leu⁺	25 Met⁺, 25 Leu⁺
	1.0	99.1	690 Met⁺, 250 Leu⁺	690 Met⁺, 530 Leu⁺
RM9 (Ile⁻ Gly Ser⁻)	0	—	50 Ile⁺, —	60 Ile⁺, —
	0.8	99.1	200 Ile⁺, —	510 Ile⁺, —

[a] Transduction mixtures contained 2.3×10^8 to 3.9×10^8 cells; multiplicities of infection are based on phage titer before UV treatment. Transductants for Trp were scored on M10C, and the others were scored on M10. When Leu or Ile was the selective marker, the amino acid was omitted from M10.

[b] Transductants for the second marker were not scored.

TABLE 6. *Transduction of Sterne strain of B. anthracis*[a]

Recipient	Transduction mixture		Colonies/ml
	Cells	PFU	
M44	5×10^8	0	25
(Trp$^-$)	5×10^8	1.2×10^9	1,460
M13	3×10^8	0	20
(Pur$^-$)	3×10^8	1.2×10^9	315

[a] Phage was propagated on wild-type Sterne strain and treated with UV to give inactivation of 97% of the PFU. The values given for PFU in transduction mixtures are based on the phage titer before UV treatment. Transductants were scored on M10C.

adsorption. In instances in which transductants for a particular marker, e.g., Trp and Pur, could be scored on either M10 or M10C, yields were usually considerably greater on M10C than on M10. These results all point to the conclusion that no single procedure was optimal for all mutants tested. The data in Table 5 illustrate differences among various mutants with respect to the influence of experimental conditions on yields of transductants.

Transduction in Other Strains

Thus far, tests for transduction have been extended only to strain 6464 among the strains of *B. cereus* and to the Sterne strain of *B. anthracis*. With strain 6464, the mutants that were tested, those requiring tryptophan or either methionine or cysteine, were transduced to prototrophy by phage propagated on wild-type 6464. In general, the frequencies of transduction with mutants of strain 6464 were somewhat lower than the frequencies obtained with mutants of 569.

Table 6 shows data on transduction of tryptophan- and purine-dependent mutants of *B. anthracis*. The frequency of transduction for the tryptophan marker was 1.2×10^{-6} per PFU and that for the purine marker was 2.6×10^{-7} per PFU.

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